Identification of novel nuclear export and nuclear localization-related signals in human heat shock cognate protein 70

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Short running title:

Novel nucleocytoplasmic signals in human Hsc70

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Abstract

Heat shock cognate protein 70 (Hsc70) serves nuclear transport of several proteins as a molecular chaperone. We have recently identified a novel variant of human Hsc70, heat shock cognate protein 54 (Hsc54) that lacks amino acid residues 464-616 in the protein binding and variable domains of Hsc70. In the present study, we examined nucleocytoplasmic localization of Hsc70 and Hsc54 using green fluorescent protein (GFP) fusions. GFP-Hsc70 is localized in both the cytoplasm and the nucleus at 37°C and accumulated into the nucleolus/nucleus after heat shock, while GFP-Hsc54 always remained exclusively in the cytoplasm in those conditions. Mutation studies indicated that 20 amino acid residues of nuclear localization-related signal (NLRS) which are missing in Hsc54 but are retained in Hsc70 are required for proper nuclear localization of Hsc70. We further found that Hsc54 contains a functional leucine–rich nuclear export signal (NES: LDVTPLSL {401}) which is differently situated from the previously proposed NES in Saccharomyces cerevisiae Ssb1p. The cytoplasmic localization of Hsc54 was impaired by a mutation in NES as well as by a nuclear export inhibitor, leptomycinB, suggesting that Hsc54 is actively exported from the nucleus to the cytoplasm through a CRM1-dependent mechanism. In contrast, the nucleocytoplasmic localization of Hsc70 was not affected by the same mutation of NES or leptomycinB. These results suggest that the NLRS could functionally mask NES leading to prolonged retention of Hsc70 in the nucleus. An additional mechanism for unmasking the NES may regulate nucleocytoplasmic trafficking of Hsc70.
**Key words:** human heat shock cognate protein 70 (Hsc70), human heat shock cognate protein 54 (Hsc54), nuclear export signal, nuclear export, leptomycinB, nucleocytoplasmic shuttling

**Introduction**

The heat shock cognate protein 70 (Hsc70), a member of the heat shock protein 70 (Hsp70) family, shuttles between the cytoplasm and the nucleus as a molecular chaperone (1-7). Hsp70/Hsc70 promotes the formation and stability of nuclear localization signal (NLS)-cargo importin-α complex and regulates the import of proteins into the nucleus *e.g.* NF-κB (8), temperature sensitive p53 (p53val-135) (9), simian virus 40 large tumor antigen (10) and nucleoplasmin (11). Under stress including heat shock and oxidative stress, Hsc70 is accumulated in the nucleolus/nucleus, which is associated with prevention of DNA damage and regulation of heat shock factor HSF1 etc. (12-14). Several mechanisms of Hsc70 nuclear import have been proposed. Hsc70 itself contains a functional nuclear targeting sequence (246KRKHKKDISENKRARR262), which has characteristics of basic nuclear localization signal (NLS) (15-16). An alternative NLS has also been claimed to be present in the amino-terminus (16). Nuclear localization of Hsc70 is dependent on the co-import of NLS-cargo (17). A functional nuclear export signal (NES) has been identified in C-terminal region of *Saccharomyces cerevisiae* Ssb1p, a homologue of human Hsc70 (18). However, human Hsc70 lacks the corresponding region to the NES in Ssb1p and therefore molecular mechanism for nuclear export of mammalian Hsc70 still remains to be uncovered.
Recently, we identified a novel variant of human Hsc70 and named it heat shock cognate protein 54 (Hsc54) (19). Hsc70 consists of three domains, the adenosine triphosphatase (ATPase) domain, the protein binding domain, and the variable domain (20-23). Hsc54 lacks 153 amino acid residues (464-616) in the protein binding and variable domains of Hsc70. In the present study, we examined nucleocytoplasmic localization of both Hsc70 and Hsc54 by constructing green fluorescent protein (GFP) fusions. By extensive mutant studies, we narrowed down the sequence required for nuclear import or export and finally defined novel NES and nuclear localization-related signal (NLRS) in Hsc70.

Materials and Methods

Plasmid construction

The pGEX-2T containing either Hsc70 or Hsc54 cDNA (19) was digested by BamHI and resulting inserts were ligated in-frame into the BamHI-BamHI or BglII-BamHI site of pEGFP-C1 (Clontech), a carboxy-terminal green fluorescent protein (GFP)-fusion vector. Several mutants of Hsc70 and Hsc54 were constructed by polymerase chain reaction-based methods using pEGFP-Hsc70 or pEGFP-Hsc54 as templates. PCR products were subcloned into pT7 Blue (Novagen) or pCR2.1-TOPO (Invitrogen) and sequenced. After cleavage with appropriate restriction enzymes, each PCR product was ligated to pEGFP-C1, pEGFP-Hsc70 or pEGFP-Hsc54, as described below. To construct internal deletion mutants of Hsc70, primers 70F4D GGAGGTGGCACTTTTGAT (sense) and Hsc70R, ACTTGGTGGCTTAATCAACC (antisense) were used. Additional primers were used for each deletions as follows:
70DD1F, AAGTTTGAACCTCACATCTATGCCTTCAAC (sense) and 70DD1R, GTTGAAGGCATAGGATGTGAGTTCAAACTT (antisense) for Del1 (∆463-543); 70DD2F, AAGAATTCATCTGAGATGCCAGAGGAATG (sense) and 70DD2R, CATTCCCTCTGGCATCTCAAGTAATCTTT (antisense) for Del2 (∆544-616); 70DD3F, AAGTTTGAACCTCACACTAATGACAAG (sense) and 70DD3R, CTTGTCACTATTGTTGAGTTCAAACTT (antisense) for Del3 (∆463-502); 7del3BF, GAGAAACATCTTACCTCTAGTCCCTCAAC (sense) and 7del3BR, GTGSSGGCSTSGGSTSSSTCTTGTCT (antisense) for Del3B (∆503-543); 70DD3FF1, AAGTTTGAACCTCACACAGATTGAAGTCACT (sense) and 70DD3FR1, AGTGACTTCAATCTGTGAGTTCAAACTT (antisense) for Del 3-1 (∆463-472); 70DD3FF2, GTCTCTGCTGTGCCACATCATAATGACAAG (sense) and 70DD3FR2, CTTGTCTTTAGTGATGTCCACAGCAGAGAC (antisense) for Del 3-2 (∆493-502); 7del3-5F, CCCCGAGGTGTTCCTAATGGTATACTCAAT (sense) and 7del3-5R, TTAGAGTATACCATTAGGAACACCTCGGGG (antisense) for Del 3-5 (∆473-482); 7del3-7F, TTTGACATTGATGCCAAGAGTAGCAGGGAAAA (sense) and 7del3-7R, TTTTCCCGTACTCTTTGGAATCAATTTCAAA (antisense) for Del 3-7 (∆483-492).

The HincII-BamHI fragment obtained from each PCR product was ligated to the HincII-BamHI site of pEGFP-Hsc70. To construct Del3+C-NLRS, primers 1470F, GGCATACCTCCTGCACC (sense), 1589STOBamR, GGATCCCTTAAGTAATCTTGTCTTCTTTTCC (antisense with artificial stop codon and BamHI site) 2007-1470F, ATTGAAGAGGTGTGAGCTCCCTCCCTGCA (sense) and 2007-1470R, TGGCAGGAGGTAGCCATCAACCTCTCTT (antisense) were used. The BamHI-EcoRI fragment obtained from the PCR product was ligated to the BamHI-EcoRI site of pEGFP-Del3. To construct Del4 (385-646), primers 70B1236F, TAAGGATCCCTCTGAGAATGGTCAAGATT (sense, with an artificial
BamHI site) and 1722R, AGGCATGGACTCAAGTGAA (antisense) were used. The BamHI-EcoRI fragment obtained from the PCR product was ligated to the BamHI-EcoRI site of pEGFP-Hsc70. To construct 54C (385-646, Δ464-616), primers 70B1236F and Hsc70R, ACTTGGTTGGCTTAACTCAACC (antisense) were used. The BamHI-BamHI fragment obtained from the PCR product was ligated to the BamHI-BamHI site of pEGFP-C1. To construct Del33 (1-462), primers Hsc792FH, ATGGTCAACCCTTTATTTGAC (sense) and Hsc1470R, CTGTGAGTCAAACCTTGC (antisense) were used. The HincII-EcoRI fragment obtained from the PCR product was ligated to the HincII-EcoRI site of pEGFP-Hsc70. To construct 13R (1-410), 89R (1-402) and 74R (1-397), sense primer Hsc792FH and each antisense primer with an artificial KpnI site, 70K1313R, ATGTTACCATGACTCCACCAGCATG (for 13R); 70K1289R, ATGTTACCAAGGGAAAGGAGGAGT (for 89R); 70K1274R, ATGTTACCAAGTGACATCAAGAGGCAG (for 74R) were used. The HincII-KpnI fragment obtained from each PCR product was ligated to the HincII-KpnI site of pEGFP-Hsc70. To construct several NES mutants of 89R, primers 70FD4 and each antisense primer were used as follows; Hsc391LA1280R, AAGGAGGAGTGCATCCAGAGCAGGCATC and Hsc1289BamR, ATGTTACCAAGGGAAAGGAGGAGTGCATC for L391A; Hsc392LA1283R, GGAAAGGAGGAGTGCATCCAGAGGCAC for L392A; Hsc393LA1283R, GGAAAGGAGGAGTGCATCCAGAGGCAC and Hsc1289BamR for L393A; Hsc394LA1289R, GGAAAGGAGGAGTGCATCCAGAGGCAC for L394A; Hsc396LA1289BamR, ATGTTACCAAGGGAAAGGAGGAGTGCATC (with BamHI site) for L396A; Hsc398LA1289BamR,
ATTGGATCCACCAAGGGAAAGAGCAGTGAC (with BamHI site) for L398A; Hsc399LA1289BamR, ATTGGATCCACCAAGGGAAAGCAGTGAC (with BamHI site) for L399A; Hsc401LA1289BamR, ATTGGATCCACCAAGGGAAAGCAGTGAC (with BamHI site) for L401A. The AccI-BamHI fragment obtained from each PCR product was ligated to the AccI-BamHI site of pEGFP-Hsc54. To construct the L399A mutant of Hsc70, primers Hsc731AccF, CTTTGAGGTCAAGTCTACAGC (sense), Hsc1449AccR, GAGACATTGAGTATACCATTGG (antisense), Hsc399LAF GGATGTCACACTCTGCTTCCCTGTTG (sense) and Hsc399LA1289R were used. The AccI-AccI fragment obtained from PCR was ligated to the AccI-AccI site of pEGFP-Hsc70. To construct L399A mutant of Hsc54, primers Hsc731AccF, pEGFP-R, ACCTCTACAAATGTGGTATGG, Hsc399LAF and Hsc399LA1289R were used. The AccI-BamHI fragment obtained from PCR was ligated to the AccI-BamHI site of pEGFP-Hsc54.

**Cell culture and transfection**

COS7 cells were cultured in DMEM supplemented with 4.5 mg/ml glucose, 10% FBS, and 100 mg/ml kanamycin in tissue culture dishes under a humidified 5% CO2, 95% air atmosphere at 37°C. Cells were transfected with pEGFP-C1 containing Hsc70, Hsc54 or mutant cDNAs using Superfect™ Transfection Reagent (QIAGEN) under the conditions recommended by the supplier. Twenty-four hours after transfection, the cells were remained in a CO2 incubator at 37°C or placed at 43°C. In some experiments, the cells were incubated with 5 ng/ml leptomycinB (LMB, SIGMA).
Nucleocytoplasmic localization of GFP-fusion proteins

Nucleocytoplasmic localization of GFP-fusion proteins was examined by direct fluorescent microscopy (Olympus IX70). The fluorescent microscope images were obtained with a CCD camera (Hamamatsu Photonics, Hamamatsu, Japan) and visualized using Mac Aspect software (Mitani, Tokyo, Japan). For evaluation of localization of GFP-fusion proteins, the relative nuclear and cytoplasmic fluorescence in >100 cells per condition from three independent experiments were determined and quantified according to a 5-grade nuclear localization score: exclusive nucleolar/nuclear fluorescence (+2); nucleolar/nuclear fluorescence exceeding cytoplasmic fluorescence (+1); equivalent nuclear and cytoplasmic fluorescence (0); cytoplasmic fluorescence exceeding nuclear fluorescence (-1); and exclusive cytoplasmic fluorescence (-2).

Results

Subcellular localization of Hsc54

Subcellular localization of Hsc54 and Hsc70 was examined using COS7 cells transfected with GFP-fusion protein expression plasmids. GFP-Hsc70 was localized in both the cytoplasm and the nucleus at 37°C, and accumulated in the nucleolus/nucleus after heat shock at 43°C for 2 h, as previously reported (12-14) (Fig. 1). In contrast, GFP-Hsc54 remained exclusively in the cytoplasm in either of those conditions. These results suggest that the sequence of residues 464-616 that is missing in Hsc54 is required for Hsc70 to accomplish nucleolar/nuclear localization.
Identification of amino acid residues required for nuclear localization of Hsc70

To identify the region essential for the nuclear localization of Hsc70, we constructed a series of GFP-fused Hsc70 with mutations within the residues 464-616. As shown in Fig. 2, Del 1 (Δ463-543) was localized predominantly in the cytoplasm, whereas Del2 (Δ544-616) was in both the cytoplasm and the nucleus. The deleted region of Del1 was then divided into two regions: Del3 (Δ463-502) and Del3B (Δ503-543). Del3 was still localized in only the cytoplasm, while Del3B was localized in both the cytoplasm and the nucleus. The deleted region of Del3 was further divided into four regions: Del3-1 (Δ463-472), Del3-5 (Δ473-482), Del3-7 (Δ483-492) and Del3-2 (Δ493-502). Among these deletions, Del3-5 and Del3-7 were localized predominantly in the cytoplasm, while Del3-1 and Del3-2 in both the cytoplasm and the nucleus. Heat shock did not affect the subcellular localization of any of those mutants. Therefore, Hsc70 requires the 20 amino acids (473 QIEVTFDIDANGILNVSAVD 492) for proper nuclear localization both at 37°C and after heat shock. There are no classical NLS sequences present in this stretch of 20 amino acids and we named it nuclear localization-related signal (NLRS). Molecular re-attachment of the deleted amino acid residues (463-502) containing NLRS to the carboxy-terminal end of Del3 (Del3+C-NLRS) partially increased its nuclear localization but failed to allow for heat shock-induced nuclear accumulation (Fig. 2). Thus, NLRS is necessary but not sufficient for heat-induced nuclear accumulation and needs to be appropriately positioned within the molecule Hsc70 for its function.

LMB induces nuclear accumulation of Hsc54
Lack of the NLRS in Hsc54 explains its cytoplasmic localization, but we also assumed an active export from the nucleus. We examined the effects of a nuclear export inhibitor LMB (24-28) on the subcellular localization of Hsc70, Hsc54, Del3-5, Del3-7 and Del3+C-NLRS. As shown in Fig. 3, LMB markedly induced nuclear translocation of GFP-Hsc54. The nuclear localization scores of GFP-Del3-5, GFP-Del3-7 (partial increase) and Del3+C-NLRS were also increased by LMB treatment. However, heat shock-induced nucleolar/nuclear accumulation of these molecules was not detected even when nuclear export was inhibited. Nucleocytoplasmic localization of GFP-Hsc70 at 37°C and heat shock-induced nucleolar/nuclear localization were not affected by LMB treatment.

**Identification of the NES-like sequence in Hsc54**

By eyeball scanning of Hsc54 amino acid sequences, we found a stretch of amino acid residues (391-401) similar to the leucine-rich NES-like sequence. An amino-terminal truncation mutant [Del4 (385-646)] that contains both the NES-like sequence and the NLRS, was localized equally in the cytoplasm and the nucleus (Fig. 4). When the residues 464-616 were further deleted from Del4 [54C (385-463 + 617-646)], the mutant was localized predominantly in the cytoplasm. Several carboxy-terminal truncations were further examined. The mutants still carrying the NES-like sequence [Del33 (1-462), 13R (1-410) and 89R (1-402)] were localized predominantly in the cytoplasm and significantly translocated into nucleus by LMB treatment as represented by 89R [Fig. 4 (B)]. In contrast, 74R (1-397) lacking a portion of the NES-like sequence was localized in both the cytoplasm and the nucleus even in the absence of LMB.
**Determination of minimum NES sequence of Hsc54**

To confirm the presence of the NES motif, we generated NES mutants substituting alanines (A) for leucines (L), valine (V) or proline (P) in the putative NES of Del89R which lacks the NLRS (Fig. 5). Introducing a point mutation of L394A (leucine at 394 was replaced with alanine), V396A, L399A or L401A, allowed nuclear localization, while other mutants, L391A, L392A, L393A and P398A, did not change the subcellular localization. A point mutation (L399A) of Hsc70 or Hsc54 was also created. The L399A mutant of Hsc54 was localized in both the cytoplasm and the nucleus. However, subcellular localization of Hsc70 was not affected by introducing the L399A mutation. The determined NES sequence is homologous to the characterized NES in several proteins including protein kinase A inhibitor, fragile X mental retardation protein, aryl hydrocarbon receptor and mitogen-activated protein kinase kinase, and is found to be highly conserved in the Hsp70 families of widely divergent species including human inducible Hsp70, human mitochondria Hsp70, bovine Hsp70, hamster BiP and E.coli DnaK (29-35).

**Discussion**

In the present study, we found that Hsc54 is localized exclusively in the cytoplasm both at 37°C and after heat shock. The cytoplasmic localization of Hsc54 may be resulted from impaired nuclear import and/or from active nuclear export. By mutational analyses of Hsc70 in the region missing in Hsc54, we determined 20 amino acid residues (473-492) of NLRS that are necessary for proper nuclear localization of Hsc70. The nuclear export inhibitor LMB induced nuclear accumulation of Hsc54, suggesting that Hsc54 is
capable of import to the nucleus as well as export to the cytoplasm through a CRM1-dependent mechanism. By mutant studies of leucine-rich NES-like sequence found in Hsc54, we finally determined a novel and functional NES \( ^{394} \text{LDVTPLSL}^{403} \). Due to the presence of this NES and absence of NLRS, dominant nuclear export over nuclear import may contribute to the largely cytoplasmic localization of Hsc54.

The core sequence of the Hsc54 NES contains both conserved spacing and hydrophobicity that fulfil the criteria of leucine-rich sequence established for NES (35). Interestingly, this NES sequence is highly conserved in Hsp70 family proteins (29-34), but is differently situated from the previously proposed NES in \textit{Saccharomyces cerevisiae} (18). Although biological significance of NES on the Hsp70 family proteins that are localized to mitochondria (hmHsp70) and endoplasmic reticulum (ham BiP) has not been established, evolutionary conservation of the NES motif makes us speculate that it may be associated with relocalization of molecules into appropriate cellular compartments (Fig. 5). Subcellular localization of Hsc70 was not affected by point mutations in NES or by treatment with LMB. Deletion mutants of the previously reported amino-terminal NLS can translocate into the nucleus (16). An additional regulatory mechanism may exist in the regulation of Hsc70 nuclear export as well as the import. A crystal structure analysis of Hsc70 has revealed that both NES and NLRS locate in the protein binding domain which has an unusual structural topology and binds to substrate proteins (36-37). LMB-induced nuclear translocation of NLRS-lacking mutants, Del3-5 and Del3-7, suggests that NLRS itself or a molecule bound to NLRS functionally masks NES resulting in nuclear retention of Hsc70 (Fig. 6). Re-attachment of NLRS to the carboxy-terminal end of the NLRS-lacking mutant, Del3, partially increased its nuclear localization. Thus, an appropriate distance between NLRS and NES may be required for NLRS to exert full function, such as NES inhibition.
Nuclear translocation of Hsc70 is dependent on the coimport of NLS-cargo (17) and/or its own NLS in the ATPase domain (15-16). It is unlikely for Hsc54 to interact with NLS-cargo because it lacks a large portion of the protein binding domain. However, Hsc54 may be capable of nuclear translocation via previously assumed NLS in Hsc70 (15-16). The molecular mechanism of heat shock-induced nucleolar/nuclear accumulation of Hsc70 has not been known. The carboxy-terminus of Hsc70 was shown to be necessary for proper nucleolar localization after heat shock (38). We have observed that Del4 which still retains both NES and NLRS is resistant to heat shock-induced nuclear accumulation [data not shown, see Fig. 4 (A)]. In addition, not only deletions of NLRS but also even subtle deletion mutations around NLRS confer on Hsc70 resistance to heat shock-induced nuclear accumulation (Fig. 2). It suggests that NLRS is necessary but not sufficient for this biological process.

Hsc70 mediates not only protein translocation across nuclear membrane but also diverse cellular process, including protein folding, refolding, assembly and disassembly. Similar to Hsc70, Hsc54 is constitutively expressed and upregulated by heat shock but may function as an inhibitory regulator of Hsc70 (19). We showed here that Hsc54 is positively exported from the nucleus to the cytoplasm and may function in the cytoplasm and/or transiently in nucleus. Identification of sequence elements that participate in nucleocytoplasmic trafficking of Hsc70/54 could help further investigation of those proteins in biology.

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**References**


**Figure legends**

**Fig. 1 Nucleocytoplasmic localization of Hsc54.** (A) Schematic structure of Hsc54 protein. Hsc70 consists of three domains, including the amino-terminal 44-kDa ATPase domain (residues 1-384), the middle 18-kDa protein binding domain (residues 385-543), and the carboxy-terminal 10-kDa variable domain (residues 544-646). (B), (C) Subcellular localization of Hsc70 and Hsc54. COS7 cells were transfected with pEGFP-C1 containing Hsc70 or Hsc54 cDNA. Twenty-four hours after transfection, subcellular localization of GFP-fusion proteins was examined by direct fluorescent microscopy. Control cells were maintained in a CO2 incubator at 37°C and heat shock cells were incubated at 43°C for 2 h. (B) Nuclear localization scores. The values represent mean ± S.D. of the scores. (C) Representative fluorescent micrographs showing fluorescence of GFP-Hsc70 and -Hsc54.

**Fig. 2 Determination of NLRS in Hsc70.** COS7 cells were transfected with pEGFP containing each of the Hsc70 mutant cDNAs. Twenty-four hours after transfection, subcellular localization of GFP-fusion proteins was examined at 37°C or after heat
shock at 43°C for 2 h. (A) Schematic structure of Hsc70, Hsc54 and Hsc70 mutants. (B) Nuclear localization scores. The values represent mean ± S.D. of the scores. (C) Representative photographs.

**Fig. 3 Effects of LMB on nucleocytoplasmic localization of GFP-Hsc70, -Hsc54, and -NLRS mutants.** COS7 cells were transfected with pEGFP containing Hsc70, Hsc54, Del3-5, Del3-7 or Del3+C-NLRS cDNA. Twenty-four hours after transfection, the cells were incubated with LMB (5 ng/ml) for 6 h at 37°C and were then heat shocked at 43°C for 2 h. Subcellular localization of GFP-fusion proteins was examined just before and after heat shock. (A) Nuclear localization scores. The values represent mean ± S.D. of the scores. (B) Representative photographs.

**Fig. 4 Identification of the NES-like sequence in Hsc54.** COS7 cells were transfected with pEGFP-containing each of the Hsc70 mutant cDNAs. Twenty-four hours after transfection, subcellular localization of GFP-fusion proteins was examined at 37°C. The cells transfected with GFP-89R were incubated with LMB (5 ng/ml) for 6 h (89R-LMB). (A) Schematic structure of Hsc70 mutants. (B) Nuclear localization scores. The values represent mean ± S.D. of the scores. (C) Representative photographs.

**Fig. 5 Determination of the NES sequence in Hsc54.** (A) NES mutants by substituting alanines for leucine, valine or proline in Del89R (L391A, L392A, L393A, L394A, V396A, P398A, L399A, L401A) and L399A mutants of Hsc54 (Hsc54L399A) and Hsc70 (Hsc70L399A). Determined NES residues were underlined. (B), (C) Subcellular localization of NES mutants. COS7 cells were transfected with pEGFP containing each of the NES mutant cDNAs. Twenty-four hours after transfection, subcellular
localization of GFP-fusion proteins was examined at 37°C. Nuclear localization scores (B). The values represent mean ± S.D. of the scores. Representative photographs (C).

(D) Comparison of the determined NES of human Hsc54 with the homologous regions of several proteins and the deduced consensus sequence. Hsp70 family proteins [human inducible (h), human mitochondria (hm) and bovine (bov) Hsp70, hamster (ham) BiP and E.coli DnaK] and NES of other proteins [protein kinase A inhibitor (PKI), fragile X mental retardation protein (FMRP), aryl hydrocarbon receptor (AhR) and mitogen-activated protein kinase kinase (MAPKK)].

Fig. 6 Hypothesized functional relationships among NLRS, NES and previously reported NLS. Hsc70 is supposed to primarily utilize NLRS for nuclear import. Heat shock-induced nucleolar/nuclear translocation of Hsc70 is also NLRS-dependent but NLRS is not sufficient for this process. However, in the absence of NLRS, NLS is to be utilized. NLRS is also supposed to functionally inhibit NES. Abrogation of NLRS therefore releases the suppression leading to active nuclear export of Hsc70.
Fig. 1
Fig. 2
Fig. 3

(A) Nuclear localization score

(B) LMB (+) vs. Heat shock

[Graph and images showing localization of Hsc70, Hsc54, Del 3-5, Del 3-7, Del 3+C-NLRS under LMB and LMB + Heat shock conditions]
Fig. 4

(A) ATPase domain
variable domain
protein binding domain

Hsc70 (1-646)
Del 4 (385-646)
54 C (385-463+617-646)
Del 33 (1-462)
13R (1-410)
89R (1-402)
74R (1-397)

(NLRSNES)

(B) Nuclear localization score

-2 -1 0 +1

Del 4 54 C Del 33 13R 89R 74R 89R-LMB

(C) Del 4 54C Del 33 13R 89R 74R 89R-LMB
(A) NES consensus sequence:

\[
\text{Hsc54/70:} \quad \begin{array}{cccccccc}
L & L & L & L & D & V & T & P & L & S & L & G \\
\downarrow & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow \\
\end{array}
\]

74R 89R


(B) Nuclear localization score

\[
\begin{array}{cccccc}
& -2 & -1 & 0 & 1 & 2 \\
\downarrow & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow \\
& & & & & \\
L399A & L401A & Hsc70 L399A & Hsc54 L399A & & \\
\end{array}
\]


(C)NES consensus sequence:

\[
\begin{array}{cccccccc}
L & L & L & X & L & X & L & X \\
\end{array}
\]


(D) NES consensus sequence:

\[
\begin{array}{cccccccc}
L & L & L & L & X & L & X & L \\
\end{array}
\]

Fig. 5
Fig. 6
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